Points to Consider, ORDA, NIH

"Gene Therapy of Canavan Disease using AAV for Brain Gene Transfer"

APPENDIX M

Scientific Abstract

This proposal, for central nervous system gene transfer using recombinant human aspartoacylase packaged in an adeno-associated virus (AAV) vector, is a logical extension of our previous gene therapy protocol for treatment of patients with Canavan Disease. The rationale is that Canavan Disease, a progressive neurodegenerative childhood disorder, may be safely and effectively treated using gene transfer methods. At present the detailed pathophysiology of Canavan Disease (CD) is still poorly defined, but the genetic basis of the disease is well established. CD is an autosomal recessive disease of chromosome 17p13, in which the gene coding for the enzyme aspartylacylase is defective. The lack of a functional enzyme leads to an increase in the substrate molecule, N-acetyl aspartate (NAA), which through undefined cellular mechanisms causes demyelination and spongiform degeneration of the brain. The natural course of the disease in untreated children is irreversible brain damage and death within the first decade of life. There is no alternative treatment for the disease, which is uniformly fatal.

Previous studies have established that delivery of human aspartoacylase cDNA using an AAV-based plasmid/condensed lipid vector (LPD) is safe and is associated with some promising clinical signs (Leone et. al., 1999; Leone et al. 2000 in press). However, gene transfer technology has advanced considerably in the last two years, and we believe that LPD is no longer the most effective method for brain gene transfer in humans. We now propose to use the AAV virion for more efficient ASPA gene delivery. High levels of expression are possible in neural cells (Freese et. al., 1997; Klein et. al., 1998; Chen et. al., 1998) using rAAV, making it among the most promising vectors for CNS gene delivery in humans. Of equal importance, rAAV has been shown to be extremely safe in pre-clinical animal studies (Kaplitt et al., 1994; During et al. 1998) and in two ongoing Phase I/II clinical trials for hemophilia and cystic fibrosis.

In our previous gene delivery system we used a viral-based promoter (CMV), which we found provided reasonable levels of basal expression in the brain. However the CMV promoter is not ideal because viral promoters are weaker than endogenous neural

promoters, and also appear to be downregulated *in vivo*. We now propose to use a much more powerful expression cassette in a viral-based AAV gene transfer system, incorporating the neural-specific enolase (NSE) promoter and post-regulatory elements. Using the NSE promoter, we have observed superior gene expression compared to the levels obtained using LPD, and we hypothesize that it will translate into greater clinical improvement. The primary objective of this study is to obtain sufficient gene transfer to raise ASPA in the brain and affect the course of the disease process, and also to prove the safety of the AAV-ASPA vector in the human brain. The production of functional ASPA enzyme is predicted to lower levels of the ASPA substrate, NAA, which has been implicated in the pathogenesis of the disease. Associated with this biochemical change, we expect to be able to detect changes in the clinical course of the disease. The pre- and post-delivery assessment will be very similar to the first clinical protocol, involving non-invasive radiological, biochemical, neurological, physiological and cognitive tests. We believe that this gene therapy trial using AAV in patients affected by Canavan Disease is likely to have an impact in the clinical treatment and disease management of this and other neurogenetic diseases.